Electrode tip localization in rats using various CT imaging techniques and BlockFace is accurate, fast and cheap as compared to histology.

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Introduction

As brain implants such as electrodes to record and stimulate neural tissue in laboratory animals are becoming more and more sophisticated, implant tip localization methods have not evolved over the last century. Even nowadays, histology and copying to stereotactic atlases remains not only the gold standard but also the most commonly used method for implant tip localization, despite huge advances in laboratory animal imaging technology. We aim to compare various modalities for electrode tip localization in terms of accuracy, time and costs.

Material and Methods

In 289g male Wistar (SD 7.8g; n=12) and 424g male Sprague-Dawley (SD 6.2; n=12) rats, preoperative CT imaging was followed by stereotactic implantation of 2 electrodes (one in each hemisphere). Next, after in vivo postoperative CT imaging (CTinvivo), unilateral electrolytic marking of the electrode tip position and euthanasia was performed. Now, an ex vivo postoperative CT with the skull and electrodes in place (CTexvivo) was followed by a 14-day iodine immersion and a new CT with and without skull and electrodes in place (CTiodine+skull; CTiodine-skull, respectively) in half of the specimens. Finally, all specimens underwent BlockFace, which is a 3D reconstruction of photographic images acquired with a digital camera facing the remainder of the paraffin block on the microtome, and histology. Six different researchers with different levels of experience picked the electrode tips either directly (when the electrode was directly visible) or indirectly via different definitions (when only the electrode track was visible).

For co-registration of the images to the Johnson-Paxinos MR-atlas, we first constructed a CT atlas based on the preoperative images of the Wistar rats. The postoperative CTs with bony anatomy were co-registered to this CT-atlas and via this atlas to the MR-atlas, while the modalities without bony anatomy were co-registered directly to the MR-atlas.

Results

All CT modalities and BlockFace allowed for electrode tip localization, with modality-specific advantages and disadvantages as compared to histology. While CTinvivo, CTexvivo and CTiodine+skull permit direct electrode visualization, CTioine-skull, BlockFace and histology only show the electrode track, that can be wider and deeper than the final electrode tip position. Depending on the target, histology and CTiodine-skull can show internal brain structures surrounding the electrode tip/trace, whereas the other modalities cannot and consequently heavily rely on the co-registration quality. Clearly, all but CTinvivo only permit localization after euthanasia, hereby only allowing subject exclusion due to erroneous implantation after completion of all tests.

Electrode tips could be reliably localized both in rats identical to and different from those used to create atlases (289g male Wistar rats and 424g male Sprague-Dawley rats, respectively).

Average time needed for localization of 2 electrodes in 1 rat brain (anesthesia, acquisition, preparation of chemicals, euthanasia and perfusion, brain extraction, paraffin embedding, slicing, staining, imaging post processing, tip picking) ranged from 27 minutes (CTinvivo) to 94 minutes (histology).

Average costs, as charged in our institution, for localization of 2 electrodes in 1 rat brain (anesthesia, CT use, chemicals and consumables, histological processing, microtome use) ranged from 5.50 euro (CTexvivo) to 21,24 euro (histology).

Conclusion

We conclude that CT imaging techniques and BlockFace are valuable alternatives to histology for electrode tip localization, and are both faster and cheaper as compared to histology.